

CLAIMS:

1. A process for producing recombinant calf-chymosin which comprises the steps of isolating calf-chymosin gene, cloning the same in bacterial expression vector PET21b, transforming said cloned vector into cells of E.coli, fermenting said E.coli strains to produce pro-chymosin, converting said pro-chymosin to chymosin and subsequently recovering the recombinant calf-chymosin.
2. The process as claimed in claim 1, wherein calf-chymosin gene is obtained by isolating RNA from the fourth stomach of calf tissue, synthesising a first strand of cDNA therefrom by treating the same with a reverse primer such as 5'-TGT GGG GAG AGT GAG GTT CTT GGT C-3' and then with a forward primer such as 5'-ATG AGG TGT CTC GTG GTG CTA CTT 3 and with a reverse primer such as 5'TGT GGT GAC AGT GAG GTT CTT GGT C-3'.
3. The process as claimed in claims 1 and 2 wherein said C DNA is ligated at small site of pBSSK+ plasmid and then transformed into TOP 10 cells of E-coli.
4. The process as claimed in claim 3 wherein said recombinant clones were identified and treated with a forward primer such as 5'-GAT ATA CAT ATG GCT AGC ATC ACT AGG ATC CCT CTG TAC 3' and reverse primer such as 5' GCA GTA AGC TTG ACA GTG TTC CTT GGT CAG CG-3' containing Nde I and Hind III sites to obtain an amplified fragment.
5. The process as claimed in claim 4 wherein said amplified fragment is transformed into cells of E.coli for expressing said chymosin gene.

6. The process as claimed in any of the preceding claims wherein said E.coli cells containing recombinant calf chymosin gene is fermented in a medium containing 12g/L peptone, 24g/L of yeast extract and 10g/L of sodium chloride in the presence of supplements for fermentation and the suspended cells produced on completion of fermentation is lysed, chilled and pH adjusted to 8 before incubating at room temperature and the supernatant containing prochymosin is separated.
7. The process as claimed in claim 6, wherein the pH of said prochymosin containing supernatant is adjusted to 2 at room temperature and further incubated for about 6 hrs with gentle stirring and filtered.
8. The process as claimed in claim 7 wherein the pH of said filtrate is adjusted to about 5 and further incubated, filtered and treated with a solution containing sodium benzoate and thereafter a solution containing and sodium chloride to activate prochymosin to chymosin.
9. The process as claimed in claim 8 wherein the filtrate obtained after the addition of sodium benzoate solution is treated with a solution of sodium chloride under stirring and cooking, and the precipitate suspended in a chilled solution of 0.2M glycine with 0.001M EDTA and thereafter treated with 0.23% solution of sodium benzoate and stored under cooling.
10. The process as claimed in claim 9 wherein said chymosin obtained is formulated with 10% of sodium chloride and 0.2% of Trehalose.

11. Recombinant calf-chymosin having the following amino acid sequence:

MetAlaSerIle ThrArgIle ProLeuTyr LysGlyLysSer LeuArgLys AlaLeuLys
 1 ATGGCTAGCA TCACTAGGAT CCCTCTGTAC AAAGGCAAGT CTCTGAGGAA GGCGCTGAAG
 TACCGATCGT AGTGATCCTA GGGAGACATG TTTCCGTTCA GAGACTCCTT CCGCGACTTC
 GluHisGlyLeu LeuGluAsp PheLeuGln LysGlnGlnTyr GlyIleSer SerLysTyr
 61 GAGCATGGGC TTCTGGAGGA CTTCTGCAG AACAGCAGT ATGGCATCAG CAGCAAGTAC
 CTCGTACCGG AAGACCTCCT GAAGGACGTC TTTGTCGTCA TACCGTAGTC GTCGTTCATG
 SerGlyPheGly GluValAla SerValPro LeuThrAsnTyr LeuAspSer GlnTyrPhe
 121 TCCGGCTTCG GGGAGGTGGC CAGCGTGCCC CTGACCAACT ACCTGGATAG TCAGTACTTT
 AGGCCGAAGC CCCTCCACCG GTCGCACGGG GACTGGTTGA TGGACCTATC AGTCATGAAA
 GlyLysIleTyr LeuGlyThr ProProGln GluPheThrVal LeuPheAsp ThrGlySer
 181 GGGAAGATCT ACCTCGGGAC CCCGCCCCAG GAGTTCACCG TGCTGTTTGA CACTGGCTCC
 CCCTTCTAGA TGGAGCCCTG GGGCGGGGTC CTCAAGTGGC ACGACAAACT GTGACCGAGG
 SerAspPheTrp ValProSer IleTyrCys LysSerAsnAla CysLysAsn HisGlnArg
 241 TCTGACTTCT GGGTACCCTC TATCTACTGC AAGAGCAATG CCTGCAAAAA CCACCAGCGC
 AGACTGAAGA CCCATGGGAG ATAGATGACG TTCTCGTTAC GGACGTTTTT GGTGGTCGCG
 PheAspProArg LysSerSer ThrPheGln AsnLeuGlyLys ProLeuSer IleHisTyr
 301 TTCGACCCGA GAAAGTCGTC CACCTTCCAG AACCTGGGCA AGCCCCTGTC TATCCACTAC
 AAGCTGGGCT CTTTCAGCAG GTGGAAGGTC TTGGACCCGT TCGGGGACAG ATAGGTGATG
 GlyThrGlyLys MetGlnGly IleLeuGly TyrAspThrVal ThrValSer AsnIleVal
 361 GGGACAGGCA AGATGCAGGG GATCCTGGGC TATGACACCG TCACTGTCTC CAACATTGTG
 CCCTGTCCGT TCTACGTCCC CTAGGACCCG ATACTGTGGC AGTGACAGAG GTTGTAAACAC
 AspIleGlnGln ThrValVal LeuSerThr GlnGluProGly AspValPhe ThrTyrAla
 421 GACATCCAGC AGACAGTAGT CCTGAGCACC CAGGAGCCCC GGGACGTCTT CACCTATGCC
 CTGTAGGTCG TCTGTCATCA GGACTCGTGG GTCCTCGGGC CCCTGCAGAA GTGGATACGG
 GluPheAspGly IleLeuGly MetAlaTyr ProSerLeuAla SerGluVal LeuAspThr
 481 GAATTCGACG GGATCCTGGG GATGGCGTAC CCCTCGCTGG CCTCAGAAGT ACTCGATACC
 CTTAAGCTGC CTTAGGACCC CTACCGCATG GGGAGCGACC GGAGTCTTCA TGAGCTATGG
 GlyPheAspAsn MetMetAsn ArgHisLeu ValAlaGlnAsp ValPheSer ValTyrMet
 541 GGCTTTGACA ACATGATGAA CAGGCACCTG GTGGCCCAAG ACGTGTTCTC GGTTTACATG
 CCGAAACTGT TGTACTACTT GTCCGTGGAC CACCGGGTTC TGCACAAGAG CCAAATGTAC
 AspArgAsnGly GlnGlyAsn MetPheThr LeuGlyAlaIle AspProSer TyrTyrThr
 601 GACAGGAATG GGCAGGGAAA CATGTTTACC CTGGGGGCCA TCGACCCGTC CTACTACACA
 CTGTCCTTAC CCGTCCCTTT GTACAAATGG GACCCCCGGT AGCTGGGCAG GATGATGTGT
 GlySerLeuHis TrpValPro ValThrVal GlnGlnTyrTrp GlnPheThr ValAspSer
 661 GGGTCCCTGC ACTGGGTGCC CGTGACAGTG CAGCAGTACT GGCAGTTCAC TGTGGACAGT
 CCCAGGGACG TGACCCACGG GCACTGTCAC GTCGTCATGA CCGTCAAGTG ACACCTGTCA
 ValThrIleSer GlyValVal ValAlaCys GluGlyGlyCys GlnAlaIle LeuAspThr
 721 GTCACCATCA GCGGTGTGGT TGTGGCCTGT GAGGGTGGCT GTCAGGCCAT CCTGGACACG
 CAGTGGTAGT CGCCACACCA ACACCGGACA CTCCCACCGA CAGTCCGGTA GGACCTGTGC
 GlyThrSerLys LeuValGly ProSerSer AspIleLeuAsn IleGlnGln AlaIleGly
 781 GGCACCTCCA AGCTGGTCGG GCCCAGCAGC GACATCCTCA ACATCCAGCA GGCCATTGGA
 CCGTGGAGGT TCGACCAGCC CGGGTCGTCT CTGTAGGAGT TGTAGGTCGT CCGGTAACCT
 AlaThrGlnAsn GlnTyrAsp GluPheAsp IleAspCysAsp AsnLeuSer TyrMetPro
 841 GCCACACAGA ACCAGTACGA TGAGTTTGAC ATCAGACTGCG ACAACCTGAG CTACATGCC
 CCGTGTGTCT TGGTCATGCT ACTCAAACCTG TAGCTGACGC TGTGGACTC GATGTACGGG
 ThrValValPhe GluIleAsn GlyLysMet TyrProLeuThr ProSerAla TyrThrSer
 901 ACTGTGGTCT TTGAGATCAA TGGCAAAATG TACCCACTGA CCCCCTCCGC CTATACCAGC
 TGACACCAGA AACTCTAGTT ACCGTTTTAC ATGGGTGACT GGGGGAGGCG GATATGGTCG
 GlnAspGlnGly PheCysThr SerGlyPhe GlnSerGluAsn HisSerGln LysTrpIle

961 CAGGACCAGG GCTTCTGTAC CAGTGGCTTC CAGAGTGAAA ATCATTCCCA GAAATGGATC
GTCCTGGTCC CGAAGACATG GTCACCGAAG GTCTCACTTT TAGTAAGGGT CTTTACCTAG
LeuGlyAspVal PheIleArg GluTyrTyr SerValPheAsp ArgAlaAsn AsnLeuVal
1021 CTGGGGGATG TTTTCATCCG AGAGTATTAC AGCGTCTTTG ACAGGGCCAA CAACCTCGTG
GACCCCTAC AAAAGTAGGC TCTCATAATG TCGCAGAAAC TGTCCCGGT GTTGGAGCAC
GlyLeuAlaLys, AlaIle***
1081 GGGCTGGCCA AAGCCATCTG A
CCCGACCGGT TTCGGTAGAC T

13. Recombinant calf-chymosin when produced by a process according to any of the preceding claims.